Jl S. Afr. Bot. 36 (2): 63–68 (1970)

CULTURE OF HAWORTHIA INFLORESCENCES IN VITRO

S. K. Majumdar

(*Department of Biology, Lafayette College, Easton, Pennsylvania, U.S.A.*)

Abstract

Leafy shoots, roots and callus are induced from the flower axes on inflorescences of four species of *Haworthia* when inflorescences are implanted in White's basal medium supplemented with indole acetic acid (1AA), coconut milk (CM) and kinetin. The callus subsequently differentiated into shoots and roots in this medium. A combination of 1AA, CM and kinetin is essential for the production of vegetative buds and callus from the flower axis. On subculturing, the callus did not grow in White's medium alone but on supplementing it with 1AA or CM or kinetin the callus differentiated into many plantlets. The addition of kinetin to the medium, however, greatly increased the production of leafy shoots but suppressed root formation.

Introduction

In recent years the artificial production of plantlets from isolated roots, stems, and floral primordia has been demonstrated (Danckwardt-Lilliestrom, 1957; Sastri, 1962; Konar and Nataraja, 1964). However, very little is known about the growth potentiality of flower axes in culture. With this in mind, tissue culture work with *Haworthia* inflorescences was started at the University of Kentucky and a partial success of this study was presented at the annual meeting of the Botanical Society of America (Majumdar and Sabharwal, 1968). This investigation was continued further by the author at the Arkansas Polytechnic College. The present report is the detailed account of the results obtained from the experiments with the inflorescences of several species of *Haworthia* grown on a variety of media.

Material and method

Haworthia plants were provided by Dr. Herbert P. Riley of the University of Kentucky who obtained them from South Africa and the International Succulent Institute in California. Successful results are obtained with four plants—*H. variegata*, small form; *H. chlorocantha*; *H. turgida* var. *pallidifolia* and *H. retusa*. The inflorescences were surface sterilized in chlorine water, washed in sterile distilled water and planted on a White's basal medium containing sucrose (2 per cent) + indole acetic acid (1AA, 1 mg/litre) + kinetin (0·5 mg/litre) + coconut milk (CM, 20%) + vitamins (pyridoxine hydro-

---

Accepted for publication 12th November, 1969.

chloride 0·1 mg/litre, nicotinic acid 0·5 mg/litre and aneurin hydrochloride 0·1 mg/litre).

Later inflorescences were inoculated on the following media: (*a*) White's basal medium with two per cent sucrose (WB) + vitamins; (*b*) WB + 1AA; (*c*) WB + CM; (*d*) WB + kinetin; (*e*) WB + 1AA + CM; (*f*) WB + 1AA + kinetin; (*g*) WB + CM + kinetin; (*h*) WB + 1AA + CM + kinetin. Ten cultures were made for each treatment and each experiment was repeated twice.

The medium was jelled with 0·8 per cent Difco bactoagar; its pH was adjusted to 5·8, and the cultures were kept at 26° ± 1°C.

OBSERVATIONS AND DISCUSSION

Inflorescences did not show any response and dried when inoculated in (*a*), (*b*), (*c*) and (*d*) media. The presence of 1AA + CM or 1AA + kinetin in

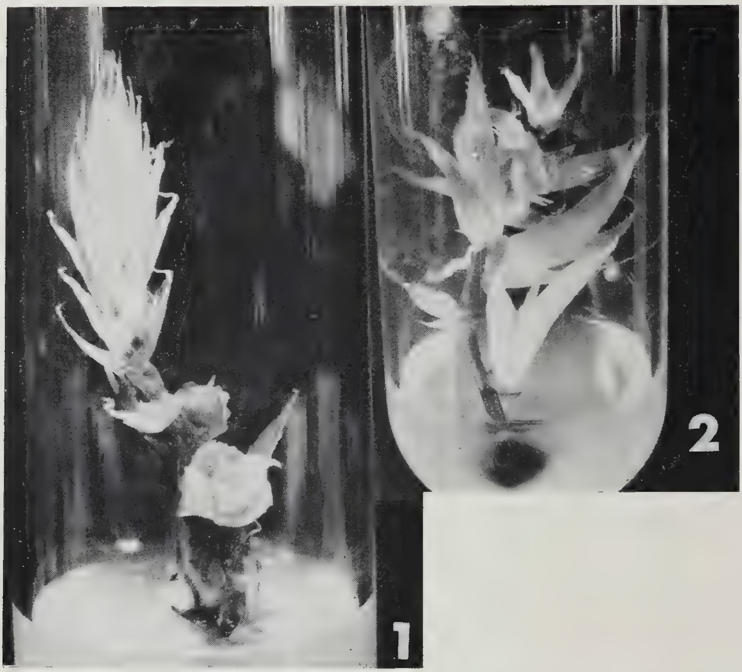
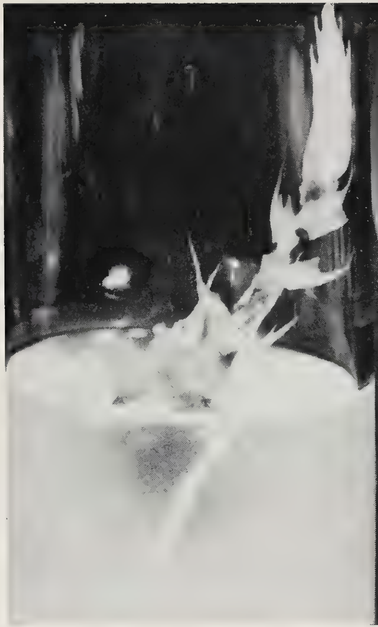


FIG. 1. *H. retusa* inflorescence showing formation of vegetative buds; note the dried flowers at the upper portion of the inflorescence, X4.

FIG. 2. *H. chlorocantha* with spiny leaves which arose from the flower axes on inflorescence, X4.



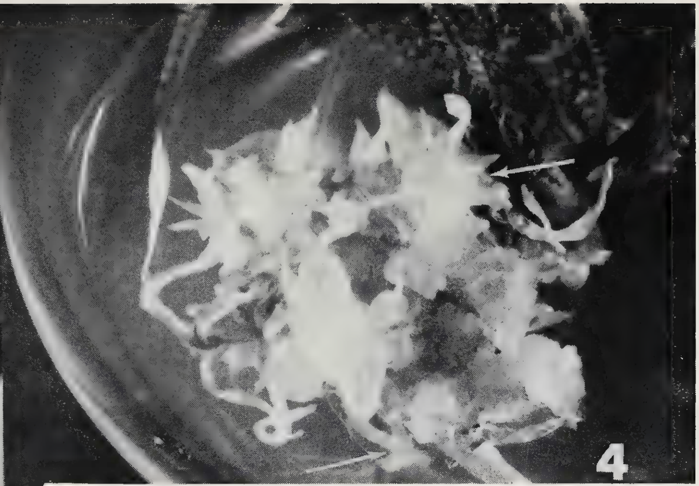


FIG. 3. *H. turgida* var. *pollidifolia* inflorescence showing callus formation from flower axis, also note the production of leafy buds and root from flower axes, X4.

FIG. 4. *H. variegata* small form, showing the differentiation of callus tissue into leafy shoots and roots (arrows), note the matured leaves which arose from a flower axis of the inflorescence, X4.

White's basic medium did not have any effect. Some response was observed when kinetin was added in the presence of coconut milk. In this medium few inflorescences produced vegetative buds from some flower axes. Very good response was observed when White's basic medium was supplemented with 1AA + kinetin + CM. In most instances the flowers on the inflorescences became dry and in about 25—30 days vegetative buds were seen to appear from some flower axes (Fig. 1). Within a few days the buds developed into leaves with spiny margins (Fig. 2) and looked like plantlets. After the formation of the leafy shoots an occasionally healthy root was found to emerge from the flower axis (Fig. 3). White callus formation was noticed almost simultaneously from flower axes of some inflorescences touching the medium (Fig. 3). At the beginning the callus growth was rapid and in about 15—20 days it differentiated into many plantlets with shoots and roots (Fig. 4). It can be seen from the figures that no healthy vegetative buds are present in the flower axes of the apical region of the inflorescences, but active growth is noticed in the lateral buds of flower axes. The lateral shoot initiation may be due to the lack of influence of apical dominance.

On subculturing, the callus differentiated into shoots and roots on all media except in WB + vitamin. However, the presence of kinetin greatly increased the production and growth of the plantlets but suppressed rooting. Many plantlets from culture tubes have been successfully transferred to soil.

Frequently flower buds of *H. variegata* grew into mature flowers or produced fasciated flowers from flower axes; a detailed account of flower bud development in *Haworthia* will be reported elsewhere (Majumdar, 1969).

It is seen that induction of vegetative buds and formation of callus from the flower axes of *Haworthia* and callus differentiation are possible when 1AA, CM and kinetin are added to the White's basal medium. However, none of the above substances alone is effective in producing vegetative buds or callus and differentiation of the callus. The absence of vitamin did not have any adverse effect on the formation of buds and callus and callus differentiation.

It is observed in subculture experiments that any one of the above mentioned chemicals (1AA, CM, kinetin) can differentiate the callus into leafy shoots with few roots. Kinetin was not indispensable for the differentiation of callus, but its presence no doubt increased the rate of the expression of the shoot morphogenesis.

This study, thus, demonstrates for the first time that the cells of the flower axis of *Haworthia* have the potentiality to produce leafy shoots and roots; and callus from the flower axis can undergo developmental sequence to reconstitute new plants.

Uittreksel

DIE KWEEK VAN *HAWORTHIA* BLOEIWYSIES IN VITRO. Steggies met blare, wortels en kallus weefsel word verkry van die blomstele uit die bloeiwyses van vier soorte *Haworthia* waar die bloeiwyses in White se basale medium, aangevul met indolasynsuur (1AA), kokosneut melk (CM) en kinetien, ingeplant is. Die kallus het in hierdie medium differensieer in stingels en wortels. 'n Kombinasie van 1AA, CM en kinetien is essensieel vir die ontwikkeling van vegetatiewe knoppe en kallus van die blomsteel. In 'n sub-kultuur het die kallus nie in White se medium alleen ontwikkel nie maar met die byvoeging van 1AA en CM of kinetien het dit in 'n menigte klein plantjies differensieer. Kinetien het egter 'n groot produksie van blaarryke stingels en 'n onderdrukte wortel vorming veroorsaak.

Acknowledgement

I thank Dr. P. S. Sabharwal of the University of Kentucky for the laboratory facilities. I am grateful to Professor Herbert P. Riley of the same institute for helpful criticisms.

References

Danckwardt-Lilliestrom, C. (1957). Kinetin induced shoot formation from isolated roots of *Isatis tinctoria. Physiol. Plantarum* **10,** 794—797.

Konar, R. N. and K. Nataraja. (1964). *In vitro* control of floral morphogenesis in *Ranunculus sceleratus* L. *Phytomorphology* **14,** 558—563.

Majumdar, S. K. and P. S. Sabharwal. (1968). Induction of vegetative buds of inflorescence of *Haworthia in vitro. Am. J. Botany* **55,** 705. (Abstract).

Majumdar, S. K. (1970). *In vitro* culture of flower buds of *Haworthia* and *Astroloba.* (Phyton 27).

Sastri, R. L. N. (1962). In plant tissue and organ culture—a symposium, edited by Maheshwari, P., Rangaswamy, N. S. (Intern. Soc. Plant. Morph. Delhi, 1962).